```
+1    Met Glu Ser Ile Ile Ile Val Gly Ala Gly Thr Phe Gly Leu Ser Thr Ala Leu Gln Leu Ala Arg Asp Gly Tyr Lys Asn Ile Lys Cys
1     ATGGAGTCGA TAATTATAGT TGGTGCCGGT ACTTTTGGGC TTTCCACAGC CTTACAGCTT GCCAGAGATG GATACAAGAA CATAAAATGT
+1    Phe Asp Lys Phe Pro Val Pro Ser Glu Ile Ala Ala Gly Asn Asp Ser Asn Lys Ile Phe His Tyr Asp Tyr Val Ala Pro Leu Ala Lys
91    TTTGACAAGT TTCCGGTTCC ATCTGAGATA GCTGCTGGAA ACGACAGTAA CAAGATTTTT CACTACGATT ATGTTGCTCC CCTGGCTAAA
+1    Pro Asn Ser Lys Glu Arg Leu Ser Leu Glu Ala Leu His Leu Trp Lys Thr Asp Pro Val Tyr Lys Pro Tyr Tyr His Pro Val Gly Phe
181   CCCAATTCAA AAGAACGGTT GAGTCTCGAA GCATTACACC TTTGGAAGAC AGATCCGGTG TACAAACCGT ACTATCATCC GGTAGGATTT
+1    Ile Leu Ala Ala Ser Ser Asp Ala Pro Leu Leu His Asp Lys Glu Tyr Tyr Glu Glu Leu Gln Lys Asn Gly Leu Arg Asn Tyr Arg Tyr
271   ATCCTGGCTG CAAGTTCCGA TGCTCCATTA CTGCATGATA AGGAATACTA TGAAGAGTTG CAAAAAAACG GACTTCGCAA TTATCGTTAT
+1    Ile Ser Thr Pro Glu Glu Phe Arg Glu Tyr Leu Pro Ile Leu Lys Gly Pro Leu Pro Asn Trp Arg Gly Tyr Val Leu Asp Gly Asp Asn
361   ATTTCAACTC CCGAGGAGTT TCGTGAGTAT TTGCCCATTT TAAAGGGCCC GTTACCCAAC TGGAGAGGAT ATGTTCTCGA CGGAGATAAC
+1    Gly Trp Leu His Ala Arg Asp Ser Leu Lys Ser Ala Tyr Glu Glu Cys Lys Arg Leu Gly Val Glu Phe Val Phe Gly Asp Asp Gly Glu
451   GGATGGTTGC ATGCTCGAGA CTCATTGAAA AGTGCATACG AAGAATGCAA ACGATTGGGA GTGGAATTTG TGTTTGGAGA CGATGGGGAA
+1    Ile Val Glu Leu Leu Asn Glu Asn Gly Lys Leu Thr Gly Ile Arg Ala Arg Ser Gly Ala Ile Phe Ser Ala Gln Lys Tyr Val Leu Ser
541   ATTGTCGAAT TACTTAACGA AAATGGAAAG TTGACGGGAA TTAGGGCCAG ATCTGGTGCC ATATTCTCGG CACAAAAATA TGTTCTCAGC
+1    Ser Gly Ala Asn Ala Val Thr Leu Leu Asn Phe Gln Arg Gln Leu Glu Gly Lys Cys Phe Thr Leu Ala His Phe Lys Val Thr Asp Glu
631   TCTGGTGCAA ATGCAGTAAC GTTGTTAAAT TTCCAGAGAC AGCTAGAAGG TAAATGTTTC ACTTTGGCAC ATTTCAAAGT GACGGATGAA
+1    Glu Ala Lys Ala Phe Lys Ser Leu Pro Val Leu Phe Asn Ala Glu Lys Gly Phe Phe Phe Glu Ala Asp Glu Asn Asn Glu Ile Lys Ile
721   GAAGCTAAAG CATTTAAAAG CTTGCCGGTC CTTTTCAATG CCGAAAAAGG GTTTTTTTTC GAGGCTGATG AAAATAACGA AATCAAAATT
+1    Cys Asn Glu Tyr Pro Gly Phe Thr His Thr Asn Glu Ser Gly Glu Ser Ile Pro Leu Tyr Arg Met Glu Ile Pro Leu Glu Ser Ala Leu
811   TGCAACGAGT ACCCTGGATT TACCCACACA AATGAATCCG GAGAGTCTAT CCCACTCTAC CGGATGGAGA TTCCACTCGA GTCAGCACTT
+1    Glu Ile Arg Gln Tyr Leu Lys Glu Thr Met Pro Gln Phe Ala Asp Arg Pro Phe Thr Lys Thr Arg Ile Cys Trp Cys Thr Asp Ser Pro
901   GAAATTAGAC AATACTTGAA AGAAACCATG CCTCAGTTTG CTGATAGACC TTTCACCAAG ACAAGAATTT GTTGGTGTAC CGACTCTCCC
+1    Asp Met Gln Leu Ile Leu Cys Thr His Pro Glu Tyr Thr Asn Leu Ile Val Ala Ser Gly Asp Ser Gly Asn Ser Phe Lys Ile Met Pro
991   GACATGCAAT TGATCTTGTG TACTCACCCA GAATACACCA ACCTTATTGT AGCATCGGGT GACTCTGGAA ATTCGTTCAA GATCATGCCA
+1    Ile Ile Gly Lys Tyr Val Ser Lys Val Val Thr Lys Gly Asp Lys Gly Leu Asp Pro Glu Asp Lys Glu Cys Trp Lys Trp Arg Pro Glu
1081  ATCATTGGCA AATATGTCAG CAAGGTTGTT ACCAAAGGTG ATAAAGGATT GGATCCGGAA GATAAAGAAT GCTGGAAATG GCGTCCTGAG
+1    Thr Trp Asp Lys Arg Gly Gln Val Arg Trp Gly Gly Arg Tyr Arg Val Ala Asp Leu Asn Glu Ile Glu Glu Trp Val Ser Val Glu Asn
1171  ACTTGGGACA AGCGGGGGCA GGTCCGCTGG GGTGGTCGAT ACCGTGTTGC GGATTTGAAC GAAATTGAAG AATGGGTTTC TGTTGAAAAT
+1    Pro Thr Pro His Lys Leu Glu ***
1261  CCCACACCAC ACAAACTAGA ATAA
```

FIG. 1

mesiiivgagtfglstalqlardgyknikcfdkfpvpseiaagndsnkifhydyvaplakpnskerlslealhlwktdpvykpyyhp
vgfilaassdapllhdkeyyeelqknglrnyryistpeefreylpilkgplpnwrgyvldgdngwlhardslksayeeckrlgvefv
fgddgeivellnengkltgirarsgaifsaqkyvlssganavtllnfqrqlegkcftlahfkvtdeeakafkslpvlfnaekgfffe
adenneikicneypgfthtnesgesiplyrmeiplesaleirqylketmpqfadrpftktricwctdspdmqlilcthpeytnliva
sgdsgnsfkimpiigkyvskvvtkgdkgldpedkecwkwrpetwdkrgqvrwggryrvadlneieewvsvenptphkle

FIG. 2

5’ -atggagtcgataattatagttggtgccggtacttttgggctttccacagccttacagcttgccagagatggatacaagaacataaaatgttttgacaagtttccggtt
ccatctgagatagctgctggaaacgacagtaacaagatttttcactacgattatgttgctcccctggctaaacccaattcaaaagaacggttgagtctcgaagcattacac
ctttggaagacagatccggtgtacaaaccgtactatcatccggtaggatttatcctggctgcaagttccgatgctccattactgcatgataaggaatactatgaagagttg
caaaaaaacggacttcgcaattatcgttatatttcaactcccgaggagtttcgtgagtatttgcccattttaaagggcccgttacccaactggagaggatatgttctcgacg
gagataacggatggttgcatgctcgagactcattgaaaagtgcatacgaagaatgcaaacgattgggagtggaatttgtgtttggagacgatggggaaattgtcgaatt
acttaacgaaaatggaaagttgacgggaattagggccagatctggtgccatattctcggcacaaaaatatgttctcagctctggtgcaaatgcagtaacgttgttaaattt
ccagagacagctagaaggtaaatgtttcactttggcacatttcaaagtgacggatgaagaagctaaagcatttaaaagcttgccggtccttttcaatgccgaaaaagggt
ttttttcgaggctgatgaaaataacgaaatcaaaatttgcaacgagtaccctggatttacccacacaaatgaatccggagagtctatcccactctaccggatggagattc
cactcgagtcagcacttgaaattagacaatacttgaaagaaaccatgcctcagtttgctgatagacctttcaccaagacaagaatttgttggtgtaccgactctcccgaca
tgcaattgatcttgtgtactcacccagaatacaccaaccttattgtagcatcgggtgactctggaaattcgttcaagatcatgccaatcattggcaaatatgtcagcaaggt
tgttaccaaaggtgataaaggattggatccggaagataaagaatgctggaaatggcgtcctgagacttgggacaagcgggggcaggtccgctggggtggtcgatac
cgtgttgcggatttgaacgaaattgaagaatgggtttctgttgaaaatcccacaccacacaaactagaataa-3’

FIG. 3

FAO－F1 5'-GGXACXTGGGGXWSXWSXACXGCXYTXCA-3'
FAO－R 2 3'- TCYTCRTYXGGYTCVAWRAARAAXCC-5'
in which ＊ **S=C+G Y=C+T R=A+G** X=A+C+G+T W=A+T V=A+C+G

FAO-F3 : 5'-ATTTCAAAGTGACGGATGAAGAAGCTAAAG-3'
adaptor primer : 3'-CGCAGTTTTCCCAGTCACGAC-5'

FAO-F 5 5'-GTGCATACGAAGAATGCAAACGATTGGGAGTGG-3'

FAO-R6 3'-CCATCCGTTATCTCCGTCGAGAACATATCCTC-5'

FAO-NcoI : 5'-ATCACCATGGAGTCGATAATTATAGTTGG-3'

FAO-XbaI : 3'-TTGATTCTAGACATGTATGTTGTAATCTTG-5'

FIG. 4

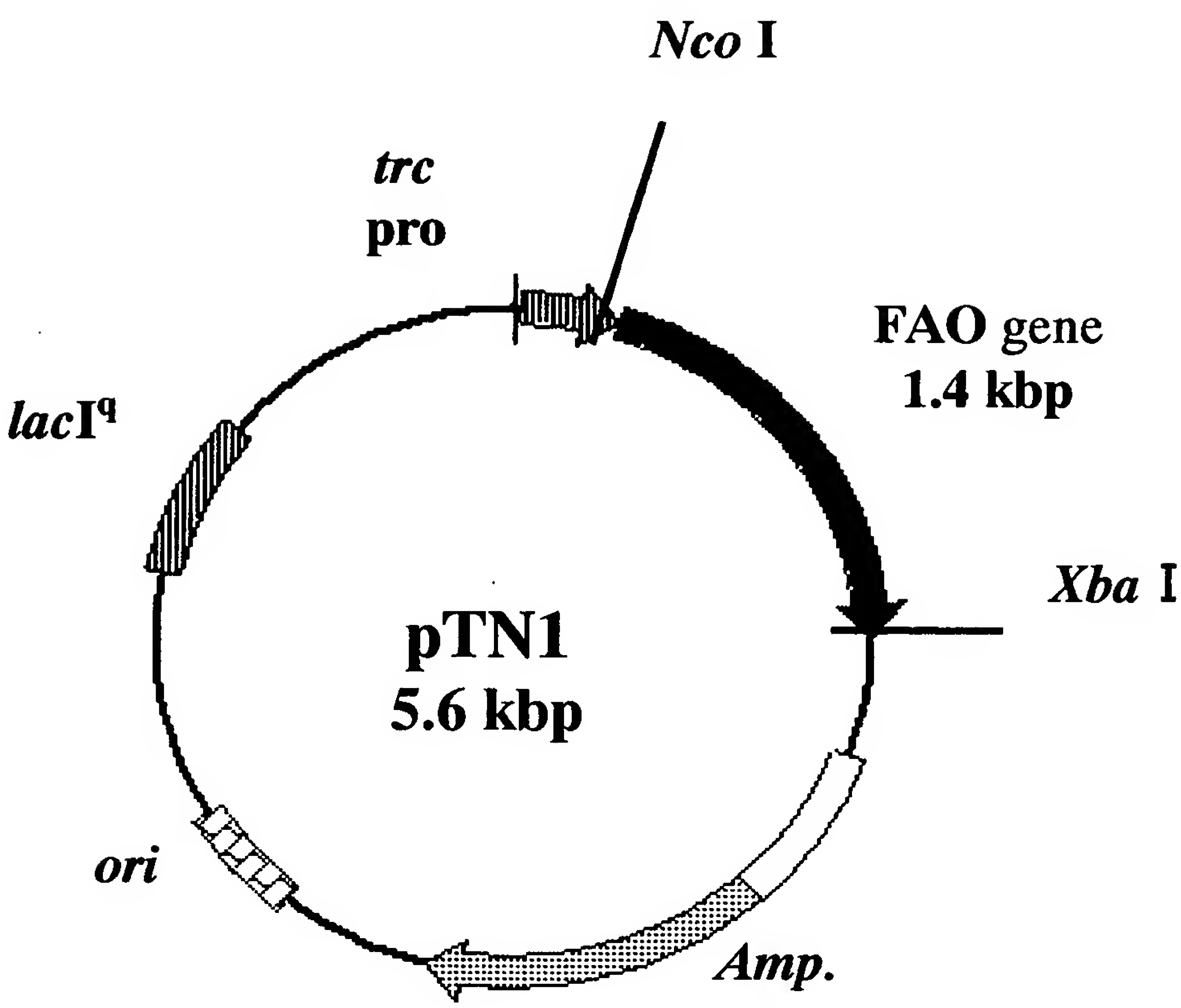
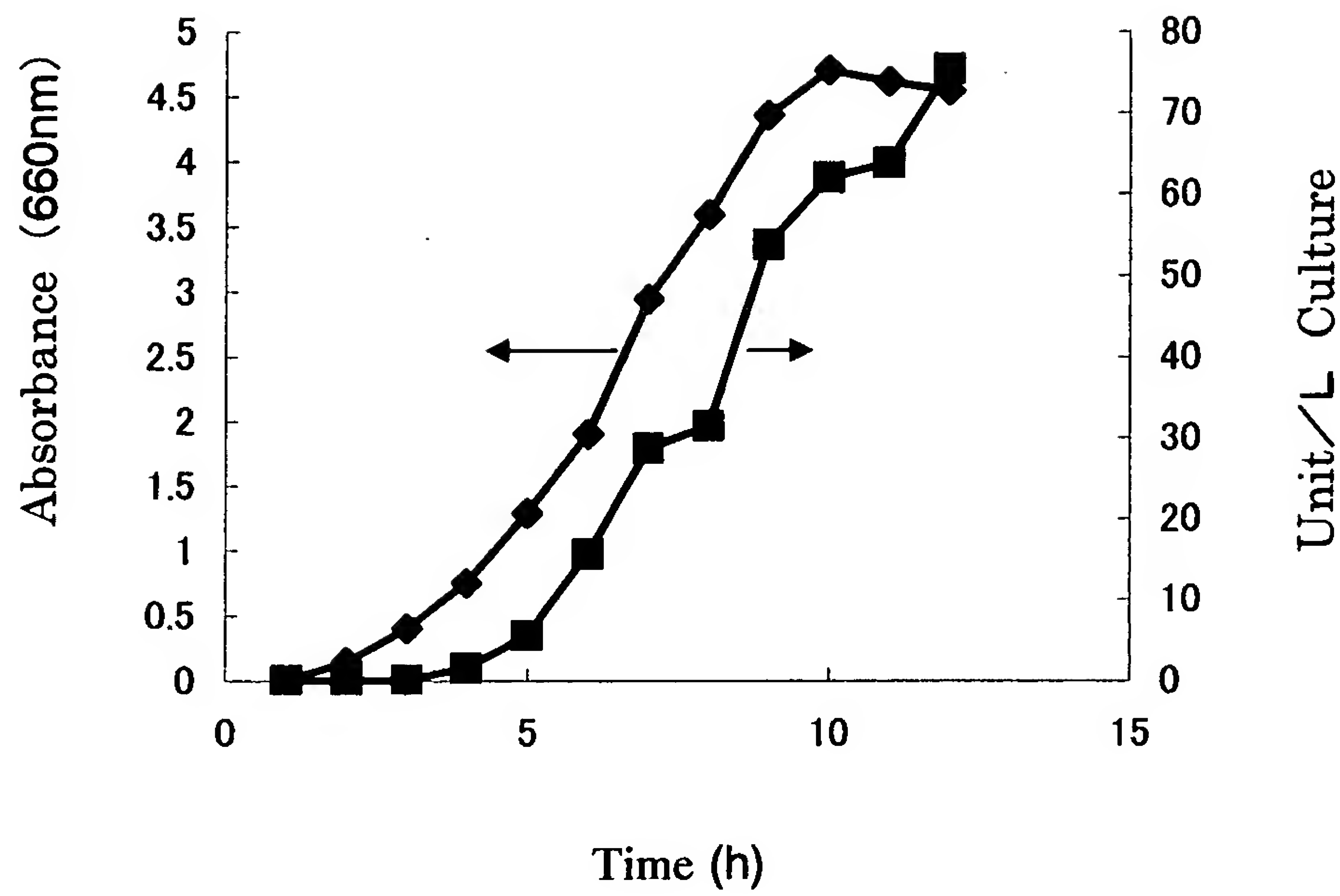


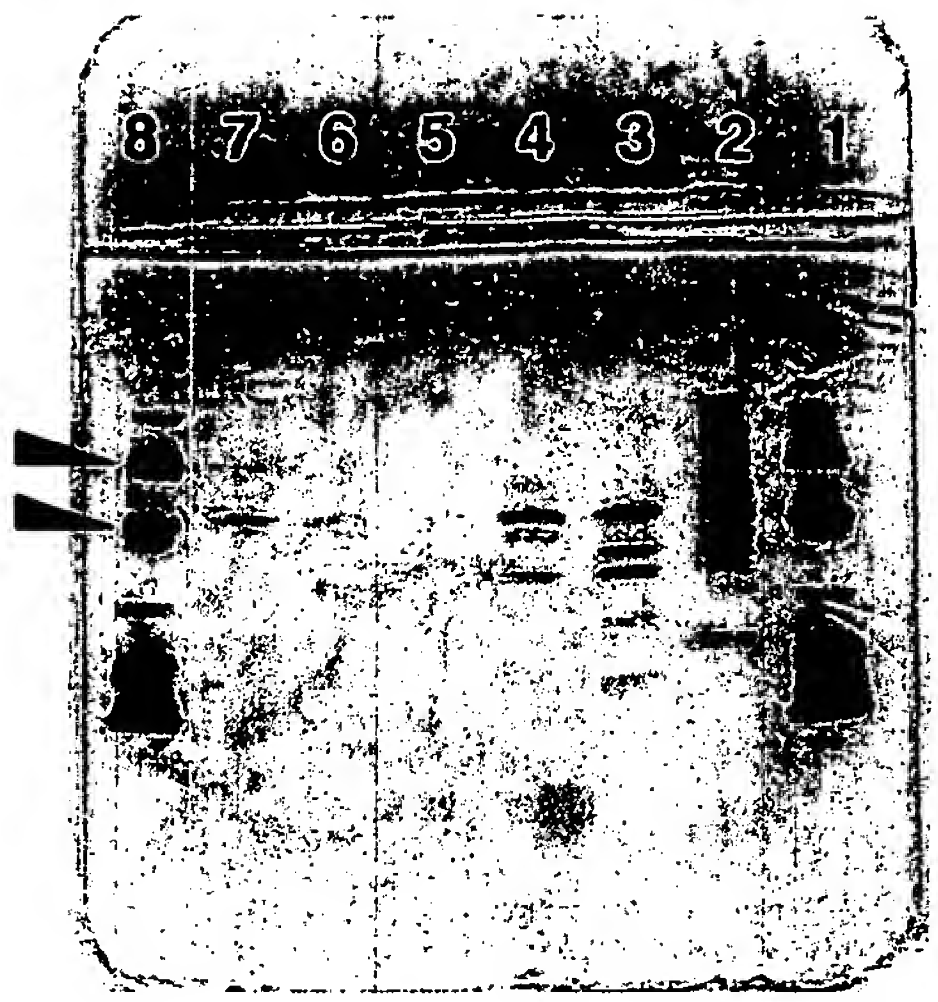
FIG. 5



5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Absorbance (660nm)
80
70
60
50
40
30
20
10
0
Unit/L Culture
0
5
10
15
Time (h)

FIG. 6

67kDa
43kDak



1: LMW
2: Soluble
3: after DEAE 5PW
4: after RESOURCE Phe
5: after Bioasist Q No.23
6: after Bioasist Q No.24
7: after Bioasist Q No.25
8: LMW

FIG. 7

Kinetic parameters

	Recombinant
Km (mM)	5.9
Vmax (U/mg)	7.1

Substrate specificity

	Activity(%)	
Substrate	**Recombinant**	**Wild type**
fructosyl valine	**100**	**100**
fructosyl lysine	**120**	**135**
fructosyl glycine	**4**	**9**
fructosyl alanine	**56**	**60**
fructosyl leusine	**14**	**31**
fructosyl phenylalanine	**104**	**103**

FIG. 8